STUDIES ON PROTEIN BINDING OF ANTIBIOTICS

V. EFFECT OF THE BINDING OF DRUG TO 100,000×*g* SUPERNATANT FLUID OF HUMAN LIVER HOMOGENATES ON URINARY EXCRETION

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To characterize the effect of the binding of antibiotics to tissue protein on urinary excretion, we examined the extent of the binding of 9 penicillins and 9 cephems to 100,000×*g* supernatant fluid of human liver homogenates.

The correlation analysis revealed a significant simple correlation between the binding to 100,000×*g* supernatant fluid of liver homogenates and urinary excretion. Drugs with lesser binding were mainly excreted in the urine, conversely, urinary excretion of highly bound drugs exhibited lower values.

The prediction with regard to urinary excretion of healthy subjects was done by multiple regression analysis using the binding to 100,000×*g* supernatant fluid and other physicochemical factors. The values predicted for urinary excretion coincided well with the observed values.

While many studies concerning drug fate have been conducted from the viewpoint of physicochemical properties of drugs using experimental animals,[1~6] the determinants by which the body directs some drugs to the bile and others to the urine is as yet unclear. In particular, the fate of drugs in the human body has yet to be elucidated. Advanced knowledge of the drug fate in humans is important from the pharmacological and toxicological point of view. Our previous study reported that binding to tissue homogenates is the major determinant in the pharmacologic behavior of antibiotics in rabbits.[7]

In this paper, therefore, the relationship between the binding to 100,000×*g* supernatant fluid of human liver homogenates and urinary excretion was investigated.

Materials and Methods

Antibiotics

The following β-lactam antibiotics were used.

Penicillins: Apalcillin (APPC, Sumitomo Chemical Co., Ltd.), piperacillin and ampicillin (PIPC and ABPC, Toyama Chemical Co., Ltd.), carbenicillin (CBPC, Taito Pfizer Co., Ltd.), sulbenicillin (SBPC, Takeda Pharmaceutical Co., Ltd.), mezlocillin (MZPC, Bayer Co., Ltd.), benzylpenicillin and oxacillin (PCG and MPIPC, Banyu Seiyaku Co., Ltd.) and ticarcillin (TIPC, Fujisawa Pharmaceutical Co., Ltd.).

Cephems: Cefoperazone (CPZ, Toyama Chemical Co., Ltd.), cefotiam and cefmenoxime (CTM and CMX, Takeda Pharmaceutical Co., Ltd.), cefmetazole (CMZ, Sankyo Co., Ltd.), cefazolin (CEZ, Fujisawa Pharmaceutical Co., Ltd.), cefoxitin (CFX, Daiichi Pharmaceutical Co., Ltd.), latamoxef (LMOX, Shionogi & Co., Ltd.), cephaloridine (CER, Torii Pharmaceutical Co., Ltd.) and cefotetan (CTT, Yamanouchi Pharmaceutical Co., Ltd.).

Preparation of 100,000×*g* Supernatant Fluid of Human Liver Homogenates

Human liver, free of any antibiotics, was removed at postmortem examination. The tissue was cut into small pieces, washed in ice-cold isotonic saline to remove as much blood as possible and homogenized in the presence of 50% (w/v) cold 1/15 M sodium phosphate buffer, pH 7.0, using a Teflon-glass motor driven homogenizer. The homogenates were first centrifuged at 24,000×*g* for 30 minutes at 4°C. The supernatant fraction was separated from the pellet and surface lipid, and then centrifuged at 100,000×*g* for 2 hours at 4°C. The supernatant fluid was pipetted off without the lipid layer and stored at −70°C. Protein was quantified by the LOWRY method,[8] using bovine albumin as the standard.

Determination of Binding Rate

To determine the binding rate of antibiotic to 100,000×*g* supernatant fluid and serum protein, the centrifugal ultrafiltration method was used. The exact procedure was described in our previous report.[7]

Reversed Phase Thin-layer Chromatography

The hydrophobic character of β-lactam antibiotics was measured by means of reversed phase thin-layer chromatography. Hydrophobicity of the tested antibiotics was expressed as the chromatographic value. The exact procedure was described in our previous report.[7]

Measurement of Antibiotic Concentration

The concentration of antibiotics, except for MZPC, TIPC and SBPC, was determined by high pressure liquid chromatography (Shimadzu LC-2). MZPC, TIPC and SBPC concentrations were determined by the paper disk method, using *B. subtilis* ATCC 6633 as a test organism.

Multiple Regression Analysis[9]

Multiple regression analysis by the method of least squares gives the following equation:

$$Y_i=\beta_0+\sum_{j=1}^{k}\beta_j X_{ij} \qquad i=1, 2, 3, \cdots n$$

where Y_i is the predicted urinary excretion of drug$_i$, X_{ij} is the factor of drug, β_j ($j=0, 1, 2, \ldots k$) the parameters to be predicted, and n is the sample number.

Urinary Excretion

Urinary excretion within 6 hours after 1 g i.v. administration, except 0.5 g i.m. administration of CER, 0.5 g i.v. of ABPC and 2 g i.v. of SBPC, were used to investigate the relationship between the binding of drug to 100,000×*g* supernatant fluid and urinary excretion.[10~15] The values used appeared to be representative for antibiotics in healthy humans.

Results

The Binding to 100,000×*g* Supernatant Fluid and Serum Protein of Drugs and Their Physicochemical Properties

Tables 1 and 2 showed the binding of 9 penicillins and 9 cephems to 100,000×*g* supernatant fluid of human liver homogenates and serum protein, as determined by the centrifugal ultrafiltration method, molecular weight, Rf value, and urinary excretion (0~6 hours).

The extent of the binding of penicillins to 100,000×*g* supernatant fluid varied from 4.0% to 37.5%. ABPC, CBPC, SBPC and TIPC were less than 10% bound, while APPC, PIPC, PCG, MZPC and MPIPC were more than 10% bound. On the other hand, the lower bound cephems, CTM, CER, CMZ, CEZ, CTZ, CFX and CMX were 5.7, 7.6, 1.0, 3.3, 4.5, 4.8 and 7.3% bound, and the higher bound drugs, CPZ and CTT, were, respectively, 18.6% and 15.5% bound to 100,000×*g* supernatant fluid. Protein concentration in the 100,000×*g* supernatant fluid used in this experiment was 3.2% to 3.6%. Serum protein binding also showed wide variation, from 20.1% for PIPC to 93.0% for MPIPC in the case of the penicillins, and, for the cephems, from 20.1% for CTM to 92.9% for CTT. CMZ, CEZ, CTZ and CMX were less bound to 100,000×*g* supernatant fluid, compared with their binding values to serum pro-

Table 1. Extent of binding of antibiotics to human liver supernatant fluid (100,000×*g*) and serum protein, Rf value, molecular weight, and urinary excretion.

Penicillins

Antibiotic	Binding (%)		Rf	MW	Urinary excretion (%) (0~6 hours)
	Liver supernatant fluid* (100,000×*g*)	Serum protein			
APPC	37.5	90.0	0	522	18.1
PIPC	22.2	20.1	0.14	518	60.5
ABPC	6.9	20.7	0.38	349	72.6
PCG	23.4	68.8	0.32	334	58.0
CBPC	7.7	52.0	0.73	378	89.0
SBPC	4.0	61.6	0.70	414	73.1
MZPC	11.8	46.8	0.40	558	60.7
TIPC	4.0	43.2	0.78	384	79.0
MPIPC	25.4	93.0	0.32	401	35.5

* Protein concentration is 3.2% to 3.6%. Mean 6-hour urine excretion after 1 g i.v. doses, except 0.5 g i.v. dose of ABPC and 2 g i.v. dose of SBPC, are used. Other definitions are described in the text.

Table 2. Extent of binding of antibiotics to human liver supernatant fluid (100,000×*g*) and serum protein, Rf value, molecular weight, and urinary excretion.

Cephems

Antibiotic	Binding (%)		Rf	MW	Urinary excretion (%) (0~6 hours)
	Liver supernatant fluid* (100,000×*g*)	Serum protein			
CPZ	18.6	89.9	0.24	646	32.6
CTM	5.7	20.1	0.19	526	73.1
CER	7.6	31.7	0.13	416	75.8
CMZ	1.0	83.3	0.58	472	84.0[a]
CEZ	3.3	92.5	0.51	455	93.9
CTZ	4.5	83.3	0.66	440	88.3
CFX	4.8	50.1	0.68	427	82.1
CMX	7.3	84.9	0.61	512	78.0[b]
CTT	15.5	92.9	0.81	576	59.5[c]

* Protein concentration is 3.2% to 3.6%. Mean 6-hour urine excretion after 1 g i.v. doses, except 0.5 g i.m. dose of CER, are used. a) Personal communication from Sankyo Co., Ltd. b) from Takeda Pharmaceutical Co. Ltd. c) from Yamanouchi Pharmaceutical Co., Ltd. Other definitions are described in the text.

tein, whereas APPC, MPIPC, CPZ and CTT, which bound more than 90% to serum protein, were shown to be more than 15% bound. The hydrophobic character of β-lactam antibiotics was expressed as an Rf value, measured by means of reversed phase thin-layer chromatography. Lower values indicate higher hydrophobicity. The Rf values were distributed, for the penicillins, from 0 for APPC to 0.78 for TIPC, and for the cephems, from 0.13 for CER to 0.81 for CTT. Molecular weight was expressed as free acid. APPC, PIPC, MZPC, CTM, CPZ, CMX and CTT are more than 500, while the others are less than 500. Urinary excretion of APPC, MPIPC and CPZ were 18.1, 35.5 and 32.6%, respectively. This indicated that these drugs were mainly excreted in the bile, because they were excreted unchanged in

Fig. 1. Relationship between binding of penicillin to 100,000×g supernatant fluid of human liver and urinary excretion.

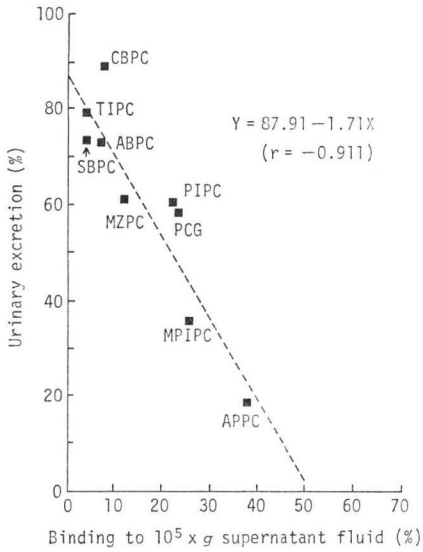
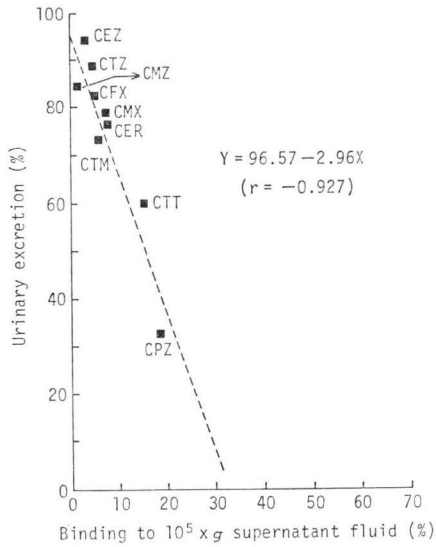


Fig. 2. Relationship between binding of cephems to 100,000×g supernatant fluid of human liver and urinary excretion.



the urine. PIPC, MZPC, PCG and CTT, whose excretions were 60.5, 60.7, 58.0 and 59.5%, respectively, were excreted moderately in the urine. Others were mainly excreted unchanged in the urine (72.6% to 93.9%).

Relationship Between the Binding to 100,000×g Supernatant Fluid of Human Liver Homogenates and Urinary Excretion

The results of the least square analysis revealed significant relationships, for both penicillins and cephems, between the binding to 100,000×g supernatant fluid and percentage of dose excreted in the urine (Figs. 1 and 2). The extent of urinary excretion tended to decrease as the binding to 100,000×g supernatant fluid increased. This relationship for both penicillins and cephems was superior to that between molecular weight and urinary excretion.

Prediction of Urinary Excretion

Urinary excretion was predicted by multiple regression analysis using the binding to 100,000×g supernatant fluid and serum protein, Rf value and molecular weight, which appear to influence drug excretion in the body. These results are shown in Tables 3 and 4. Each equation (1), which involved all factors used, was obtained for penicillins and cephems. Each equation could be further simplified by the forward selection procedure.[9] From the results of the partial F-test, equation (2), the most recently entered variable to the regression, was statistically significant. The coefficient of determination for the penicillins was 94.1%, and 85.9% for the cephems. Using each equation (2), urinary excretion were calculated. No large discrepancy was found between observed and predicted values (Tables 3 and 4).

Discussion

The fate of drugs has been studied by several investigators using rats, guinea pigs and rabbits with studies concerning molecular weight being particularly frequent. HIROM *et al.* measured the biliary ex-

Table 3. Multiple regression analysis in human. Penicillins

$Y=66.11+0.505X_1-0.541X_2+70.90X_3-0.0310X_4$ (1)
(Coefficient of determination: 95.1%)
$Y=49.26+0.553X_1-0.540X_2+77.43X_3$ (2)
(Coefficient of determination: 94.1%)
X_1 : binding to supernatant fluid (100,000×g) of human liver
X_2 : binding to serum protein
X_3 : Rf value
X_4 : molecular weight
Y : urinary excretion

Antibiotic	Urinary excretion (%)	
	Observed value	Predicted value
APPC	18.1	21.4±12.9*
PIPC	60.5	61.5±14.6
ABPC	72.6	71.3±13.2
PCG	58.0	49.8± 7.6
CBPC	89.0	82.0±11.3
SBPC	73.1	72.4±11.5
MZPC	60.7	61.5± 9.1
TIPC	79.0	88.6±11.7
MPIPC	35.5	37.9±10.7

* 95% confidence limit.

Table 4. Multiple regression analysis in human. Cephems

$Y=126.72-1.92X_1-0.0473X_2+11.20X_3-0.0942X_4$ (1)
(Coefficient of determination: 93.9%)
$Y=96.57-2.96X_1$ (2)
(Coefficient of determination: 85.9%)
X_1 : binding to supernatant fluid (100,000×g) of human liver
X_2 : binding to serum protein
X_3 : Rf value
X_4 : molecular weight
Y : urinary excretion

Antibiotic	Urinary excretion (%)	
	Observed value	Predicted value
CPZ	32.6	41.6±13.1*
CTM	73.1	79.7± 6.2
CER	75.8	74.1± 5.8
CMZ	84.0	93.6± 9.1
CEZ	93.9	86.8± 7.4
CTZ	88.3	83.3± 6.7
CFX	82.1	82.4± 6.5
CMX	78.0	75.0± 5.8
CTT	59.5	50.8±10.3

* 95% confidence limit.

cretion rate of 16 organic anions and concluded that there was a threshold molecular weight for appreciable biliary excretion: about 325±50 for rats, 400±50 for guinea pigs and 475±50 for rabbits.[1] WRIGHT *et al.* also examined the biliary excretion of 18 cephalosporin derivatives with varying 3- and 7-positions in cannulated rats, and showed that the threshold molecular weight was about 450.[3] Moreover, LEVINE speculated that a threshold molecular weight of between 500 and 600 exists for humans, although such has not actually been demonstrated.[2] Thus, it is generally recognized that the molecular weight of the compounds has a dominant influence on drug elimination. In addition, it is known that the properties of polarity, chemical structure, lipophilicity and so on are capable of influencing biliary or urinary excretion. The fate of drugs, however, cannot be predicted by the above mentioned physicochemical factors alone.

Our results demonstrated that the binding to 100,000×g supernatant fluid was also a major determinant in the fate of drugs in humans. Close attention, therefore, should be paid to the binding to 100,000×g supernatant fluid in predicting drug fate, in addition to physicochemical properties. The equation obtained was able to predict the urinary excretion of latamoxef,[19] cefbuperazone[20] and cefpiramide[21] (unpublished data). These results could be used to predict toxicological effect, and for phase I study.

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